

CLAIMS

We claim:

1. An array composition comprising:
 - a) a substrate with a surface comprising discrete sites; and
 - b) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises a bioactive agent; and
 - c) at least one fiducial;wherein said microspheres are distributed on said surface.
2. An array composition according to claim 1 wherein each subpopulation comprises a unique optical signature.
3. An array composition according to claim 2, wherein said unique optical signature is a bleed-through signature.
4. An array composition according to claim 1 wherein each subpopulation comprises an identifier binding ligand that will bind a decoder binding ligand such that the identification of the bioactive agent can be elucidated.
5. An array composition according to claim 1, wherein said substrate is selected from glass or plastic and said fiducial is at least one fiducial edge.
6. An array composition according to claim 1 wherein said substrate is a fiber optic bundle and said fiducial is a fiducial fiber.
7. An array composition according to claim 1 wherein said substrate is a fiber optic bundle, said array comprises at least three fiducials, and each of said fiducials is a fiducial fiber.
8. An array composition according to claim 7 wherein at least one of said fiducial fibers has a different shape from the others.
9. An array composition according to claim 7 wherein at least one of said fiducial fibers has a different color from the others.
10. An array composition according to claim 7 wherein at least one of said fiducial fibers has a different bleed-through signature from the others.

11. An array composition according to claim 1 wherein said fiducial is a defined edge of said substrate.

12. An array composition according to claim 1 wherein said fiducial is an interface between differing materials within said fiber optic bundle.

13. An array composition according to claim 1 wherein said fiducial is an edge of said array.

14. An array composition according to claim 1 wherein said fiducial is a microsphere.

15. An array composition according to claim 14, wherein said fiducial microsphere is distinguished from the population of non-fiducial microspheres according to its different size.

16. An array composition according to claim 1 wherein said bioactive agents are nucleic acids.

17. An array composition according to claim 1 wherein said bioactive agents are proteins.

18. An array composition according to claim 1 further comprising a computer readable memory comprising:

- a) computer code that receives a first data image; and
- b) computer code that registers said first data image using said fiducial to generate a first registered data image.

19. An array composition according to claim 18 wherein said computer readable memory further comprises:

- a) computer code that receives a second data image;
- b) computer code that registers said second data image using said fiducial to generate a second registered data image; and
- c) computer code that compares said first and said second data image.

20. A composition comprising a computer readable memory to direct a computer to function in a specified manner, said computer readable memory comprising:

- a) an acquisition module for receiving a data image of a random array comprising a plurality of discrete sites;
- b) a registration module for registering a data image; and
- c) a comparison module for comparing registered data images.

21. A composition according to claim 20 wherein said random array comprises a fiber optic bundle and said registration module utilizes a fiducial fiber for registration.

22. A composition according to claim 20 wherein said random array comprises microspheres and said registration module utilizes a fiducial microsphere for registration.

23. A composition according to claim 20 wherein said registration module utilizes a fiducial template for registration.

24. A composition according to claim 20 further comprising a random array comprising:

- a) a substrate with a surface comprising discrete sites; and
- b) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises a bioactive agent; wherein said microspheres are distributed on said surface.

25. A method of making an array composition comprising:

- a) forming a surface comprising individual sites on a substrate;
- b) distributing microspheres on said surface such that said individual sites contain microspheres, wherein said microspheres comprise at least a first and a second subpopulations each comprising a bioactive agent; and
- c) incorporating at least two fiducials onto said surface.

26. A method according to claim 25 wherein said subpopulations further comprise an identifier binding ligand that will bind a decoder binding ligand such that the identification of the bioactive agent can be elucidated.

27. A method according to claim 25 wherein said subpopulations further comprise an optical signature such that the identification of the bioactive agent can be elucidated.

28. A method according to claim 25 wherein said substrate is a fiber optic bundle and at least one of said fiducials is a fiducial fiber.

29. A method according to claim 28 wherein said fiducial fiber is a different size than other fibers.

30. A method according to claim 25 wherein said substrate is a fiber optic bundle, said array comprises at least three non-linear fiducials, and each of said fiducials is a fiducial fiber.

31. A method according to claim 30 wherein at least one of said fiducial fibers has a different shape from the others.

32. A method according to claim 30 wherein at least one of said fiducial fibers has a different size from the others.

33. A method according to claim 30 wherein at least one of said fiducial fibers has a different color from the others.

34. A method according to claim 30 wherein at least one of said fiducial fibers has a different bleed-through signature from the others.

5 35. A method according to claim 25 wherein said fiducial is a defined edge of said substrate.

36. A method according to claim 25 wherein said fiducial is a fiducial bead.

37. A method according to claim 25 wherein said bioactive agents are nucleic acids.

38. A method according to claim 25 wherein said bioactive agents are proteins.

39. A method for comparing separate data images of a random array comprising:

- 10 a) using a computer system to register a first data image of said random array to produce a registered first data image;
- b) using said computer system to register a second data image of said random array to produce a registered second data image; and
- 15 c) comparing said first and said second registered data image to determine any differences between them.

40. A method according to claim 39 wherein said random array comprises a fiber optic bundle and the registration of said first data image utilizes a fiducial fiber.

41. A method according to claim 39 wherein said random array comprises microspheres and the registration of said first data image utilizes a fiducial microsphere.

20 42. A method according to claim 39 wherein the the registration of said first data image utilizes a fiducial template.

43. A method of decoding a random array composition comprising

- a) providing a random array composition comprising:
- i) a substrate with a surface comprising discrete sites; and
- 25 ii) a population of microspheres comprising at least a first and a second subpopulation, wherein each subpopulation comprises a bioactive agent;

wherein said microspheres are distributed on said surface;

b) adding a first plurality of decoding binding ligands to said array composition and creating a first data image;

- c) using a fiducial to generate a first registered data image;
d) adding a second plurality of decoding binding ligands to said array composition and creating a second data image;
e) using said fiducial to generate a second registered data image; and
f) using a computer system to compare said first and said second registered data image to identify the location of at least two bioactive agents.

44. A method according to claim 34 wherein said random array comprises a fiber optic bundle and the registration of said first data image utilizes a fiducial fiber.

45. A method according to claim 34 wherein said random array comprises microspheres and the registration of said first data image utilizes a fiducial microsphere.

46. A method according to claim 34 wherein the registration of said first data image utilizes a fiducial template.

47. A method according to claim 34 wherein said bioactive agents are proteins.

48. A method according to claim 34 wherein said bioactive agents are nucleic acids.

49. A method of determining the presence of a target analyte in a sample comprising:

- a) acquiring a first data image of a random array composition comprising:
i) a substrate with a surface comprising discrete sites; and
ii) a population of microspheres comprising at least a first and a second subpopulation each comprising a bioactive agent;
wherein said microspheres are distributed on said surface such that said discrete sites contain microspheres;
b) registering said first data image to create a registered first data image;
c) contacting said random array composition with said sample;
d) acquiring a second data image from said array with said sample;
e) registering said second data image to create a registered second data image; and
f) comparing said first and said second registered data images to determine the presence or absence of said target analyte.

50. A method according to claim 40 wherein said random array comprises a fiber optic bundle and the registration of said first data image utilizes a fiducial fiber.

51. A method according to claim 40 wherein said random array comprises microspheres and the registration of said first data image utilizes a fiducial microsphere.

52. A method according to claim 40 wherein the the registration of said first data image utilizes a fiducial template.

53. A method according to claim 40 wherein said bioactive agents are proteins.

54. A method according to claim 40 wherein said bioactive agents are nucleic acids.

55. A method of signal pre-processing comprising:

a) acquiring a first data image of a random array composition comprising:

i) a substrate with a surface comprising discrete sites; and

ii) a population of microspheres comprising at least a first and a second subpopulation each comprising a bioactive agent;

wherein said microspheres are distributed on said surface such that said discrete sites contain microspheres;

b) determining the similarity of a first signal from at least one discrete site to at least one reference signal, wherein when said first signal is similar to at least one of said reference signals, said at least one discrete site contains a bead.

56. A method according to claim 55, wherein said determining comprises obtaining said first signal from said at least one discrete site and comparing said first signal to a threshold similarity measure obtained by comparing a reference signal to a theoretical signal, wherein when said first signal is within said threshold similarity measure, said first discrete site contains a bead.

57. A method according to claim 56 wherein when said first signal is not within said threshold similarity measure, said first discrete site does not contain a bead.

58. A method according to claim 56 wherein when said first signal is not within said threshold similarity measure, said first discrete site contains a defective bead.

59. A method according to claim 57 or 58 further comprising disregarding said discrete site wherein said first signal is not within said threshold similarity measure.

60. A method according to claim 56 wherein when said first signal is within said threshold similarity measure, said first discrete site contains a bead that comprises an optical signature that is similar to said reference signal.